PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ROLLINS, Anthony J.
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RECEIVED
11 JUL 2001

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	09.07.2001
Applicant's or agent's file reference: PL9915-PCT	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/04104	08/05/2000	17/05/1999

Applicant: LOOG, Martin et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

DUE DATE:	N/A
FORMALITIES:	SH ✓
PAT. OFF:	AK
ON DB:	12/9/01
CASE NO:	PL9915-WO

ON XL
ON DB

Name and mailing address of the IPEA:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
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CLEERE, C
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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PL9915-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/04104	08/05/2000	17/05/1999
International Patent Classification (IPC) or national classification and IPC C12N9/12		
Applicant LOOG, Martin et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/12/2000	09.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Novak, S Telephone No. +49 89 2399 8930

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/04104

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-19 as originally filed

Claims, No.:

1-14 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: OLSEN S R ET AL: 'AFFINITY PURIFICATION OF THE C-A AND C-B ISOFORMS OF THE CATALYTIC SUBUNIT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 31, 1989, pages 18662-18666, XP002150232 ISSN: 0021-9258 cited in the application

D2: SWANSON KENNETH D ET AL: 'Transcription factor phosphorylation by pp90rsk2: Identification of Fos kinase and NGFI-B kinase I as pp90rsk2.' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 6, 5 February 1999 (1999-02-05), pages 3385-3395, XP002150233 ISSN: 0021-9258 cited in the application

D3: RICOUART A ET AL: 'DESIGN OF POTENT PROTEIN KINASE INHIBITORS USING THE BISUBSTRATE APPROACH' JOURNAL OF MEDICINAL CHEMISTRY,AMERICAN CHEMICAL SOCIETY. WASHINGTON,US, vol. 34, no. 1, 1991, pages 73-78, XP002918324 ISSN: 0022-2623 cited in the application

D4: MEDZIHRADSZKY DENES ET AL: 'Solid-phase synthesis of adenosine phosphopeptides as potential bisubstrate inhibitors of protein kinases.' JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 116, no. 21, 1994, pages 9413-9419, XP002150234 ISSN: 0002-7863 cited in the application

ad V.

1. Novelty (Article 33(2) PCT)

1.1. The present application is concerned with a method for the removal of a protein kinase from a liquid. This is achieved by contacting the liquid with a bifunctional carrier bound affinity ligand, characterized by the structure:

C-(L)n-N

where

(a) C contains a structure inhibiting binding of the peptide/protein substrate to the protein kinase,
(b) L is an organic linker,
(c) n is an integer 0 or 1, and
(d) N is an inhibitor competitively inhibiting binding of the nucleoside triphosphate (NTP) to the protein kinase.

1.2. This subject-matter is not disclosed in any of the available prior art documents. Consequently, claims 1 - 14 meet the requirements as set forth in Article 33(2) PCT.

2. Inventive Step (Article 33(3) PCT)

2.1. The closest prior art is considered to result from D1.
D1 describes a synthetic peptide of 18 amino acids corresponding to the inhibitory domain of the heat-stable protein kinase inhibitor. By using this peptide in an affinity column Ca and Cb isoforms of the cAMP-dependent protein kinase were enriched 200-400-fold (see abstract).
Also encompassed by this document is a detailed description of the reagents used in this procedure, respectively the multiple steps and parameters relevant for a successful purification (see page 18662, left column).

Please note that D2 is another document concerned with a method assaying the activity of protein kinases by affinity purification using immobilized bisindolylmaleimide, which represents an ATP homolog (see page 3386, right column; page 3388).

2.2. The present application is distinguished therefrom only inasmuch the ligand used in the known method to enrich and/or purify protein kinases is characterized by the bifunctional structure "C-(L)n-N" as indicated under item 1.1.

This distinguishing feature leads to enhanced purification of the respective liquid containing said protein kinase.

The technical problem to be solved was therefore to provide a method for the

removal of protein kinase which would use alternative ligands/inhibitors displaying a higher affinity or selectivity.

2.3. For this purpose the person skilled in the art would naturally look for better (higher affinity or selectivity) inhibitors, and in consequence find the bisubstrate inhibitors, described in D3 (or alternatively D4) for the solution of this particular problem.

D3 is drawn to the design of potent protein kinase inhibitors mimicking in the same structure both the ATP binding site and a protein substrate. Considerations are also put on the use of a covalently linked spacer which could reproduce the distance between the binding sites in the enzyme structure (see page 73, left column). Table I of D3 lists the structure of 8 preferred embodiments.

D4 is another document describing the relevance of bisubstrate inhibitors of protein kinases. Also, the importance of using such bisubstrate analogs to specifically inhibit of a number of protein kinases is stressed.

2.4. It follows that the inhibitors mentioned in D3 and/or D4 essentially correspond to the features which distinguish the invention from the state of the art.
It does not matter that they were published some years earlier than the application, since their teaching is of timeless value for the person skilled in the art.
The reasoning for the obviousness is also not drawn from hindsight, but results from a clear analysis combining said documents in a straightforward way, starting from the closest prior art (D1), and looking for the solution (alternative, improved inhibitors) in further documents (D3, and/or D4).

2.5. Consequently, no inventive step can be acknowledged for the subject-matter of claims 1 and 2.

The same applies to the subject-matter of claims 3 - 14 because the additional features mentioned therein do not add any surprising and/or advantageous effect involving the effort of inventive skill, but merely represent straightforward choices of variations within said ligands.

ad VIII.

3. Clarity (Article 6 PCT)

3.1. The term "bifunctional inhibitor" used in claim 1 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

Please note that as a general rule, the area defined by the claims must be as precise as the invention allows. It is emphasised that claims must be clear on their own and that they must state the technical features which are necessary for the definition of the claimed subject-matter. Moreover, independent claims must state the essential feature of the invention.

On the basis of common general knowledge concerning the relation between structure and function, properties are predictable only within certain limits. All compounds which have been shown to solve the problem posed, share the following structural characteristics; this structural feature appears to be essential for the required activity:

C-(L)n-N

where
(a) C contains a structure inhibiting binding of the peptide/protein substrate to the protein kinase,
(b) L is an organic linker,
(c) n is an integer 0 or 1, and
(d) N is an inhibitor competitively inhibiting binding of the nucleoside triphosphate (NTP) to the protein kinase.

Consequently, present claim 1 does not satisfy the requirements a set forth in Article 6 PCT.

PATENT COOPERATION TREA

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
NYCOMED AMERSHAM PLC
Attn. ROLLINS, Anthony J.
Amersham Laboratories
White Lion Road
Bucks HP7 9LL
UNITED KINGDOM

	Date of mailing *(day/month/year)* 03/11/2000
Applicant's or agent's file reference PL9915-PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/EP 00/04104	International filing date *(day/month/year)* 08/05/2000
Applicant LOOG, Mart	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

   Where? Directly to the International Bureau of WIPO
   34, chemin des Colombettes
   1211 Geneva 20, Switzerland
   Fascimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

   Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

   Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Andria Overbeeke-Siepkes

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

NL

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 9/12, C07K 1/22 — A1

(11) International Publication Number: WO 00/70029

(43) International Publication Date: 23 November 2000 (23.11.00)

(21) International Application Number: PCT/EP00/04104

(22) International Filing Date: 8 May 2000 (08.05.00)

(30) Priority Data:
9901807-9 17 May 1999 (17.05.99) SE

(71)(72) Applicants and Inventors: LOOG, Mart [EE/EE]; Rua 11–12, EE51010 Tartu (EE). URI, Asko [EE/EE]; Paeva St. 23–2, EE50103 Tartu (EE). JARV, Jaak [EE/EE]; Wiiralti St.m 31–4, EE51010 Tartu (EE). EK, Pia [SE/SE]; Nyhagen, S–740 30 Bjorklinge (SE).

(74) Agents: ROLLINS, Anthony, John; Nycomed Amersham plc, Amersham Laboratories, White Lion Road, Amersham, Buckinghamshire HP7 9LL (GB) et al.

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR THE PURIFICATION OF PROTEIN KINASE BY AFFINITY CHROMATOGRAPHY

(57) Abstract

A method for removal of protein kinase from a liquid by contacting the liquid with a carrier bound affinity ligand for the kinase. The method is characterized in that the ligand is a bifunctional inhibitor for the kinase.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

NK

ENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PL9915-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 04104	08/05/2000	17/05/1999
Applicant		
LOOG, Mart		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets. .

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   METHOD FOR THE PURIFICATION OF PROTEIN KINASE BY AFFINITY CHROMATOGRAPHY

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/04104

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/12 C07K1/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OLSEN S R ET AL: "AFFINITY PURIFICATION OF THE C-A AND C-B ISOFORMS OF THE CATALYTIC SUBUNIT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 31, 1989, pages 18662-18666, XP002150232 ISSN: 0021-9258 cited in the application abstract page 18663, left-hand column --- -/--	1-10,14

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 October 2000	03/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lejeune, R

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SWANSON KENNETH D ET AL: "Transcription factor phosphorylation by pp90rsk2: Identification of Fos kinase and NGFI-B kinase I as pp90rsk2." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 6, 5 February 1999 (1999-02-05), pages 3385-3395, XP002150233 ISSN: 0021-9258 cited in the application page 3388 ---	1-10,14
Y	RICOUART A ET AL: "DESIGN OF POTENT PROTEIN KINASE INHIBITORS USING THE BISUBSTRATE APPROACH" JOURNAL OF MEDICINAL CHEMISTRY,AMERICAN CHEMICAL SOCIETY. WASHINGTON,US, vol. 34, no. 1, 1991, pages 73-78, XP002918324 ISSN: 0022-2623 cited in the application abstract table I ---	1-10,14
Y	MEDZIHRADSZKY DENES ET AL: "Solid-phase synthesis of adenosine phosphopeptides as potential bisubstrate inhibitors of protein kinases." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 116, no. 21, 1994, pages 9413-9419, XP002150234 ISSN: 0002-7863 cited in the application abstract ---	1-10,14
P,A	LOOG MART ET AL: "Adenosine-5'-carboxylic acid peptidyl derivatives as inhibitors of protein kinases." BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 9, no. 10, 17 May 1999 (1999-05-17), pages 1447-1452, XP004164910 ISSN: 0960-894X the whole document -----	1-14

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PL9915-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/04104	08/05/2000	17/05/1999
International Patent Classification (IPC) or national classification and IPC C12N9/12		
Applicant LOOG, Martin et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/12/2000	09.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Novak, S Telephone No. +49 89 2399 8930

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/04104

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

   1-19 as originally filed

   Claims, No.:

   1-14 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: OLSEN S R ET AL: 'AFFINITY PURIFICATION OF THE C-A AND C-B ISOFORMS OF THE CATALYTIC SUBUNIT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 31, 1989, pages 18662-18666, XP002150232 ISSN: 0021-9258 cited in the application

D2: SWANSON KENNETH D ET AL: 'Transcription factor phosphorylation by pp90rsk2: Identification of Fos kinase and NGFI-B kinase I as pp90rsk2.' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 6, 5 February 1999 (1999-02-05), pages 3385-3395, XP002150233 ISSN: 0021-9258 cited in the application

D3: RICOUART A ET AL: 'DESIGN OF POTENT PROTEIN KINASE INHIBITORS USING THE BISUBSTRATE APPROACH' JOURNAL OF MEDICINAL CHEMISTRY,AMERICAN CHEMICAL SOCIETY. WASHINGTON,US, vol. 34, no. 1, 1991, pages 73-78, XP002918324 ISSN: 0022-2623 cited in the application

D4: MEDZIHRADSZKY DENES ET AL: 'Solid-phase synthesis of adenosine phosphopeptides as potential bisubstrate inhibitors of protein kinases.' JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 116, no. 21, 1994, pages 9413-9419, XP002150234 ISSN: 0002-7863 cited in the application

ad V.

1. Novelty (Article 33(2) PCT)

1.1. The present application is concerned with a method for the removal of a protein kinase from a liquid. This is achieved by contacting the liquid with a bifunctional carrier bound affinity ligand, characterized by the structure:

C-(L)n-N

where

(a) C contains a structure inhibiting binding of the peptide/protein substrate to the protein kinase,
(b) L is an organic linker,
(c) n is an integer 0 or 1, and
(d) N is an inhibitor competitively inhibiting binding of the nucleoside triphosphat (NTP) to the protein kinase.

1.2. This subject-matter is not disclosed in any of the available prior art documents. Consequently, claims 1 - 14 meet the requirements as set forth in Article 33(2) PCT.

2. Inventive Step (Article 33(3) PCT)

2.1. The closest prior art is considered to result from D1.
D1 describes a synthetic peptide of 18 amino acids corresponding to the inhibitory domain of the heat-stable protein kinase inhibitor. By using this peptide in an affinity column Ca and Cb isoforms of the cAMP-dependent protein kinase were enriched 200-400-fold (see abstract).
Also encompassed by this document is a detailed description of the reagents used in this procedure, respectively the multiple steps and parameters relevant for a successful purification (see page 18662, left column).

Please note that D2 is another document concerned with a method assaying the activity of protein kinases by affinity purification using immobilized bisindolylmaleimide, which represents an ATP homolog (see page 3386, right column; page 3388).

2.2. The present application is distinguished therefrom only inasmuch the ligand used in the known method to enrich and/or purify protein kinases is characterized by the bifunctional structure "C-(L)n-N" as indicated under item 1.1.

This distinguishing feature leads to enhanced purification of the respective liquid containing said protein kinase.

The technical problem to be solved was therefore to provide a method for the

removal of protein kinase which would use alternative ligands/inhibitors displaying a higher affinity or selectivity.

2.3. For this purpose the person skilled in the art would naturally look for better (higher affinity or selectivity) inhibitors, and in consequence find the bisubstrate inhibitors, described in D3 (or alternatively D4) for the solution of this particular problem.

D3 is drawn to the design of potent protein kinase inhibitors mimicking in the sam structure both the ATP binding site and a protein substrate. Considerations are also put on the use of a covalently linked spacer which could reproduce the distance between the binding sites in the enzyme structure (see page 73, left column). Table I of D3 lists the structure of 8 preferred embodiments.

D4 is another document describing the relevance of bisubstrate inhibitors of protein kinases. Also, the importance of using such bisubstrate analogs to specifically inhibit of a number of protein kinases is stressed.

2.4. It follows that the inhibitors mentioned in D3 and/or D4 essentially correspond to the features which distinguish the invention from the state of the art.
It does not matter that they were published some years earlier than the application, since their teaching is of timeless value for the person skilled in the art.
The reasoning for the obviousness is also not drawn from hindsight, but results from a clear analysis combining said documents in a straightforward way, starting from the closest prior art (D1), and looking for the solution (alternative, improved inhibitors) in further documents (D3, and/or D4).

2.5. Consequently, no inventive step can be acknowledged for the subject-matter of claims 1 and 2.

The same applies to the subject-matter of claims 3 - 14 because the additional features mentioned therein do not add any surprising and/or advantageous effect involving the effort of inventive skill, but merely represent straightforward choices of variations within said ligands.

ad VIII.

3. Clarity (Article 6 PCT)

3.1. The term "bifunctional inhibitor" used in claim 1 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

Please note that as a general rule, the area defined by the claims must be as precise as the invention allows. It is emphasised that claims must be clear on their own and that they must state the technical features which are necessary for the definition of the claimed subject-matter. Moreover, independent claims must state the essential feature of the invention.

On the basis of common general knowledge concerning the relation between structure and function, properties are predictable only within certain limits. All compounds which have been shown to solve the problem posed, share the following structural characteristics; this structural feature appears to be essential for the required activity:

C-(L)n-N

where
(a) C contains a structure inhibiting binding of the peptide/protein substrate to the protein kinase,
(b) L is an organic linker,
(c) n is an integer 0 or 1, and
(d) N is an inhibitor competitively inhibiting binding of the nucleoside triphosphate (NTP) to the protein kinase.

Consequently, present claim 1 does not satisfy the requirements a set forth in Article 6 PCT.